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## Supplement Review Immunotherapy of type 1 diabetes: lessons for other autoimmune diseases

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### Chapter summary

The nonobese diabetic (NOD) mouse is a well-recognised animal model of spontaneous autoimmune insulin-dependent diabetes mellitus. The disease is T-cell mediated, involving both CD4 and CD8 cells. Its progress is controlled by a variety of regulatory T cells. An unprecedented number of immunological treatments have been assessed in this mouse strain. This chapter systematically reviews most of these therapeutic manoeuvres, discussing them in the context of their significance with regard to the underlying mechanisms and the potential clinical applications. The contrast between the surprisingly high rate of success found for a multitude of treatments (more than 160) administered early in the natural history of the disease and the few treatments active at a late stage is discussed in depth. Most of the concepts and strategies derived from this model apply to other autoimmune diseases, for which no such diversified data are available.

**Keywords:** autoimmune diseases, immunotherapy, insulin-dependent diabetes mellitus

### Introduction

Insulin-dependent diabetes mellitus (IDDM), or type 1 diabetes, is a T-cell-mediated autoimmune disease. Much effort has been devoted over the past two decades to establishing an immunological treatment that could substitute for insulin therapy. In this chapter, I provide an update of the noteworthy preclinical data obtained in the spontaneous animal models of the disease and of clinical trials in progress. These data are presented with particular attention to lessons that could benefit the immunotherapy of other autoimmune diseases, notably rheumatoid arthritis.

### IDDM as an autoimmune disease

It is now firmly established that in the vast majority of cases, IDDM has an autoimmune origin [1]. This does not preclude the possible aetiological role of a triggering envi-

ronmental factor, notably a pancreatotropic virus, but the fact remains that the  $\beta$ -cell lesion is mediated by  $\beta$ -cell-specific autoreactive T cells.

No consensus has been reached on the nature of the effector T cell(s). Research on the nonobese diabetic (NOD) mouse has shown that both CD4 and CD8 clones could induce the disease separately, but it is likely that the two cell types cooperate in the  $\beta$ -cell lesion. CD8 T cells could act through a direct cytotoxic mechanism, although this has not been proven. CD4 cells could act either as helper T cells or as effector cells through cytokine production.

Increasing importance is given to various subsets of regulatory T cells that have been shown to control the onsets of diabetes in both the NOD mouse and the BioBreeding

A glossary of specialist terms used in this chapter appears at the end of the text section.

(BB) rat. Three main types of regulatory T cell have been described [2]: Th2 cells, which appear after administration of soluble  $\beta$ -cell autoantigens, CD4<sup>+</sup>CD25<sup>+</sup> T cells, and natural killer 1 cells, which probably appear spontaneously during ontogeny. It is not yet clear whether the onset of diabetes results from the decline of T-cell-mediated regulation or, what is more likely, from the overriding of the regulation by activation of  $\beta$ -cell-specific effector T cells. Another major uncertainty relates to the nature of the events that trigger such activation. Antigen mimicry or pancreatic inflammation are the most likely, but not necessarily the only, mechanisms.

### Strengths and limitations of the NOD mouse model

More than 100 reports have been published using the NOD mouse to set up new immunotherapeutic strategies. Table 1 presents a nonexhaustive list of the main products or strategies tested so far.

The large number of successful results in this mouse has raised the question of the validation of the model as a preclinical tool for identifying strategies to be applied ultimately to humans. For several substances, the success in the NOD mouse has been confirmed in humans, e.g. cyclosporin A [3,4], heat shock protein (hsp)60 peptide [5], and anti-CD3 antibody (K Herold, unpublished observations). For others, however, such confirmation was not obtained, e.g. nicotinamide [6], oral insulin [7,8], and BCG (bacille Calmette-Guérin) [9]. It is important to realise that, contrary to human diabetes, which is essentially seen in the clinic when the disease is overt, diabetes in the NOD mouse can be studied at all stages of its natural history, including the preclinical stages. It is interesting in this context that the three drugs shown to be effective in human diabetes were still efficient in the NOD mouse at an advanced stage, whereas nicotinamide, BCG, and oral insulin worked only at the preclinical stage.

An intriguing question is whether the preventive effects observed after administration of a drug at a very early stage (e.g. 4–6 weeks of age) are specific. An attractive hypothesis is that early intervention resets the homeostasis of the immune system before the disease starts to progress irreversibly. It could be postulated that there is a checkpoint before which the disease outcome is not yet fixed. An agent that would inhibit the triggering event or boost immunoregulation could then show a long-term effect. Applied after this checkpoint, the agent would not show any significant therapeutic effect. To illustrate this concept, it may be suggested that if a virus causes the initial insult that triggers the onset of the diabetogenic process and that virus can be eliminated, an antiviral treatment could be effective if applied very early but would be ineffective once the initial inflammation had occurred and induced a sustained immune response to  $\beta$ -cell autoantigens.

The NOD mouse is one of the few spontaneous models of T-cell-mediated autoimmune diseases, and as such it is of special interest to all students of autoimmunity. This mouse strain is also of major interest because it has been used to generate many genetically modified models in which various genes have been deleted or overexpressed as transgenes in various tissues including the  $\beta$  cells (using the rat insulin promoter). Such mice provide invaluable help in discerning the mode of action of the various therapeutic strategies shown to operate in wild NOD mice.

A weakness of the NOD mouse model is that the putative target  $\beta$ -cell autoantigen(s) is (are) unknown. Several candidates have been proposed, such as glutamic acid decarboxylase, insulin, hsp60, and IA-2 [1], but no firm evidence has shown any of them to be primary autoantigens. This is not necessarily a major pitfall, since data have been accumulated to indicate that such a primary autoantigen may not exist. Even if it exists, diversification of autoimmune specificities (antigen spreading) occurs so fast that the primary antigen may not be crucial. Additionally, at the level of cytokine-dependent immunoregulation (cytokines are discussed further in section 6 below), the occurrence of bystander suppression [10] allows the suppression initially directed against a given  $\beta$ -cell antigen, whether it is a primary autoantigen or not, to be extended to most  $\beta$ -cell-specific T-cell responses.

### Preclinical studies: a unique array of approaches

As mentioned above, a wide spectrum of agents or manipulations has been shown to prevent, and more rarely to cure, IDDM in NOD mice. They are listed here according to the factors postulated to contribute to the development of the disease. The various strategies that have been reported are presented below, and Table 1 lists the reference or references relevant to each product or strategy.

#### 1. T-cell depletion or sequestration/diversion

The most straightforward approach to immunotherapy of a T-cell-mediated autoimmune disease such as IDDM is the removal of T cells, either targeted as a whole or as subsets. This has been accomplished in the NOD mouse using several approaches.

Anti-T-cell depleting antibodies offer the easiest strategy. One may thus delay the onset of diabetes by administration of depleting CD4 antibodies such as GK 1.5 and, to a lesser extent, CD8, CD44, CD45RA, or CD45RB antibodies. However, although the onset of diabetes can be prevented in the best cases, there is no clear effect on overt disease, even when it is only recently established. In recently established disease, besides anti-CD3 antibodies, which essentially act independently of major T-cell depletion (see below), only a mixture of depleting CD4 and CD8 antibodies or polyclonal antilymphocyte antibodies have been

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Table 1

## Immunotherapeutic agents or other treatments used in NOD mice

1 T-cell depletion or sequestration/diversion	6.3 IL-12
1.1 Depletion	Anti-IL-12 [80]
Anti-CD3 [28]	IL-12 antagonist (p40)2 [81]
Anti-CD4 [40]	6.4 IFN- $\alpha$ (oral) [82]
Anti-CD8 [41]	6.5 IL-1
Anti-CD44 [42]	IL-1 antibody [83]
Anti-CD45RA [43]	IL-1 antagonist [84]
Anti-CD45RB [44]	6.6 IL-6 [75]
Anti-Thy 1.2 [45]	6.7 Lymphotoxin receptor [85]
Antilymphocyte globulin [11,46]	7 Pharmacologically active cytokines
Neonatal thymectomy [46]	7.1 IL-4 [86]
1.2 Sequestration/diversion	7.2 IL-10 [87,88]
Anti-CD43 [47]	7.3 IL-13 [89]
Anti-VLA-1 [48]	7.4 IL-3 [37]
Anti-VLA-4 [48,49]	7.5 G-CSF (F Zavala, unpublished observations)
VLA-4/Ig fusion protein [50]	7.6 Lymphotoxin [90]
Anti-CD82L [49]	7.7 IL-11 [91]
2 Blockade of T-cell activation	7.8 IL-1 $\alpha$ [92]
2.1 Chemical immunosuppressants	7.9 TNF- $\alpha$ [28]
Cyclosporin A [51]	8 Tolerance to soluble $\beta$ -cell autoantigens
FK-506 [52]	8.1 Insulin
Azathioprine [53]	Oral [93]
Rapamycin [54]	Oral + IL-10 [94]
Deoxyspergualin [55]	Intranasal [34,95]
2.2 $\gamma$ Irradiation [56]	Subcutaneous
3 Targeting of T-cell receptors	Native protein [96]
3.1 TCR $\alpha\beta$ antibody [13]	B chain [96]
3.2 CD3 antibody [28]	Inactive analogue [95,97]
3.3 V $\beta$ 8 antibody [57]	DNA vaccination [98]
3.4 T-cell vaccination	Gene-transfer delivery [99] (proinsulin gene)
Polyclonal activated T cells [58]	Cholera-toxin conjugate [100]
Glutaraldehyde-treated T cells [59]	8.2 Glutamic acid decarboxylase (GAD)
Activated T cells	Oral [101]
V $\beta$ 8 T cells [60]	Intranasal [102]
Anti-hsp60 T-cell clone [61]	Subcutaneous [103]
3.5 Blocking peptides [62]	Intrathymic [104]
4 Targeting of MHC molecules	DNA vaccination [105]
4.1 Anti-class-I [63]	Anti-GAD antibody [106]
4.2 Anti-class-II [64]	8.3 Heat shock protein 60 (hsp60)
4.3 MHC transgenic mice	Subcutaneous or intraperitoneal
Class I [85]	Protein [107]
I-A [16,86]	P277 peptide [108,109]
I-E [87]	Gene-transfer delivery [110]
5 Targeting of costimulation and adhesion molecules	8.4 Pancreatic extracts (oral) [111]
5.1 Costimulation molecules	9 Stimulation of regulatory T cells
Anti-CD28 [68]	9.1 Pathogens
CTLA-4-Ig fusion protein [69]	Bacteria
Anti-B7.2 [69]	Mycobacteria
Anti-CD40L [70]	<i>Mycobacterium bovis</i> [112]
5.2 Adhesion molecules	<i>M. avium</i> [113]
Anti-ICAM-1 [71]	Complete Freund's adjuvant [114]
Soluble ICAM-1	<i>Lactobacillus casei</i> [115]
Recombinant protein [72]	Streptococcal extract [116]
Gene therapy (P Lemarchand, unpublished observations)	<i>Klebsiella</i> extract [117]
Anti-Mac [73]	<i>Escherichia coli</i> (+ oral insulin) [118]
Anti-LFA-1 [71]	Viruses
6 Cytokine blockade	Mouse hepatitis virus [119]
6.1 IFN- $\gamma$	Lactate dehydrogenase virus [120]
Anti-IFN- $\gamma$ [74,75]	Lymphocytic choriomeningitis virus [121]
IFN- $\gamma$ /IgG1 fusion protein [76]	Parasites
6.2 IL-2	Filariae [122]
Anti-IL-2R [77]	Schistosoma [123]
IL-2R/Ig fusion protein [78]	9.2 Stimulation of innate immunity
IL-2 diphtheria-toxin protein [79]	$\alpha$ -Galactosylceramide [33,124]

Continued overleaf S5

Table 1 continued

## Immunotherapeutic agents or other treatments used in NOD mice

9.3 Nondepleting anti-T-cell antibodies		12 Inhibition of $\beta$ -cell lesion	
Anti-CD3 [28]		12.1 Nicotinamide [145]	
Anti-CD4 [30]		12.2 Antioxidants	
Superantigens [125]		Vitamin E [146]	
10 Gene therapy		Probucol analog [147]	
10.1 $\beta$ -cell antigens		Probucol + deflazacort [148]	
DNA vaccination [98,105]		Aminoguanidine [149]	
GAD immunoglobulin [126]		12.3 Anti-inflammatory agents	
10.2 IL-4		Pentoxifylline [150]	
Retrovirus (T-cell transfection) [127]		Roflumilast [150]	
Bioliatic [128]		13 Miscellaneous	
Adenovirus [129]		13.1 Immunomodulators	
IL-4/IgG1 fusion protein [130]		Linomide [151]	
10.3 IL-10		Ling-zhi-8 [152]	
T-cell transfection [131]		D-Glucan [153]	
Local [132]		Multi-functional protein 14 [154]	
Systemic [133]		Ciamexon [155]	
10.4 ICAM-1 (P Lemarchand, in preparation)		Cholera toxin B [156,157]	
10.5 IFN- $\gamma$ /IgG1 fusion protein [76,130]		Vanadate [158]	
10.6 TGF- $\beta$ [134]		Vitamin D <sub>3</sub> analogue [159]	
10.7 Calcitonin [135]		13.2 Hormones and related proteins	
11 Cell therapy		Androgens [160]	
11.1 Islet or segmental pancreas transplantation		IGF-I [163]	
(+ immunosuppression)		13.3 Immunomanipulation	
Syngeneic [12]		Natural antibodies [161,162]	
Allogeneic [136] (+ immunosuppression)		Lupus idiotypic [163]	
11.2 Intrathymic islet transplantation [38]		Lipopolysaccharide [164,165]	
11.3 Bone marrow transplantation		13.4 Diet	
Allogeneic [137,138]		Casein hydrolysate [166,167]	
Syngeneic [37]		13.5 Other	
11.4 Dendritic cells [139,140]		Sulfatide [168]	
11.5 Natural killer T cells [141]		Bee venom [90]	
11.6 CD4 cell lines		Kampo formulation [169]	
Polyclonal [142]		Silica [170]	
Anti-Ia <sup>b</sup> [143]		Ganglioside [171]	
11.7 Allogeneic cells		Antiasialo GM-1 antibody [172]	
Macrophages [144]		Hyaluronidase [42]	
Spleen cells [36]		Concanavalin A [173]	

CD45RA(B), CD46 receptor A(B); CDXXL, CDXX ligand; CFA, complete Freund's adjuvant; GAD, glutamic acid decarboxylase; G-CSF, granulocyte colony-stimulating factor; ICAM-1, intercellular adhesion molecule-1; IFN, interferon; MHC, major histocompatibility complex; TCR, T-cell receptor; V, variable region (of immunoglobulin); VLA, very late antigen.

found to reverse the disease [11]. Immunosuppression is not specific to  $\beta$ -cell antigens and may be prolonged, thus exposing the patient to the hazards of generalised immunosuppression. A more subtle approach, which is probably less hazardous but also less efficient, targets T-cell homing molecules, aiming at diverting pathogenic T cells or their precursors from migrating to the islets. This is the putative mode of action of anti-VLA-1, anti-VLA-4, anti-CD43, and anti-L-selectin (CD62L) antibodies.

## 2. Blockade of T-cell activation

A less radical but similar approach to the previous one is to reversibly block T-cell activation. At present, this is achieved using chemical immunosuppressants.

Most drugs used in organ transplantation where T cells are also incriminated have been used, and these include,

notably, cyclosporin A, azathioprine, rapamycin, FK506, and deoxyspergualin. Again, these drugs essentially worked when given early in the course of the disease as a preventive, but not a curative, treatment. This point is illustrated by results reported by Wang and Lafferty and their coworkers, showing that in diabetic NOD mice transplanted with syngeneic islets, recurrence of diabetes could be prevented by a depleting CD4 antibody (GK 1.5) but not by cyclosporin A [12].

## 3. Targeting of T-cell receptors

T-cell-receptor(TCR)-mediated recognition of  $\beta$ -cell autoantigens is a central step in the diabetes pathogenesis, at both the triggering and the effector phases. It was thus logical to attempt to block TCRA. This has been successfully achieved using a number of approaches.

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Global TCR blockade can be obtained by administering antibodies directed against the constant portion of  $\alpha\beta$  TCRs or to the CD3 complex with which TCR is tightly associated both physically and functionally. In the case of CD3, though, the blockade effect is only part of the antibody mode of action, which also involves depletion (at least when the entire antibody molecule is used) and especially T-cell activation notably of regulatory T cells (see below). Here again, at least for TCR $\alpha\beta$  antibody, immunosuppression is of the global type and works only preventively. Regression of diabetes was observed in mice with recently manifested diabetes [13], which is interesting inasmuch as it provides strong support to the argument that reversible T-cell-mediated inflammation takes place in the islets. However, such regression was inconsistent and transient (at variance with that induced by anti-CD3 as described below).

A more selective approach is to target T-cell subsets using selective TCR V $\beta$  antibodies, on the assumption that pathogenic T cells preferentially use selective V $\beta$  genes. Some encouraging but as yet unconfirmed results have been reported for V $\beta$ 8.1 and V $\beta$ 6. In fact, the experimental model in which such V $\beta$  gene restrictive usage was initially reported, namely experimental allergic encephalomyelitis [14], has not been confirmed for other experimental autoimmune diseases. When whole myelin antigens are used, no clear V $\beta$  gene restrictive usage has been found in human autoimmune diseases. A special case might be made for human diabetes for V $\beta$ 7 (and perhaps V $\beta$ 13), which are seemingly preferentially used by T cells present in islet infiltrates [15].

A last and even more specific TCR blockade could be obtained by immunising against idiotypes of pathogenic T cells, ideally T-cell clones. This has been attempted in the NOD mouse either using polyclonal T cells or T-cell clones, notably clones of anti-hsp60 T cells. Some effect was reported, but the results, which were often only partial, require confirmation.

#### 4. Targeting of MHC molecules

Peptides of  $\beta$  cells are presented to T cells in the context of MHC molecules. It was thus logical to attempt to modulate the course of  $\beta$ -cell-specific autoimmunity in NOD mice targeting MHC molecules. Administration of either class-I-specific or class-II-specific monoclonal antibodies in young NOD mice (less than 2 months old) but not older ones prevents the onset of diabetes. The protection afforded by class II antibodies is long lasting and resistant to cyclophosphamide and can be transferred to nonantibody-treated mice by T cells. Its precise mode of action, however, remains elusive. It is noteworthy that NOD transgenic mice overexpressing mutated MHC non-NOD class II genes are protected from diabetes and, again, the protection can be transferred to wild NOD mice by T cells

from transgenic mice [16,17]. Collectively, these data suggest that targeting MHC molecules might lead to stimulation of regulatory class II restricted CD4 T cells, which are as yet uncharacterised.

MHC molecules could also be targeted by blocking peptide binding to those molecules; this possibility is suggested by the prevention of diabetes that is afforded by the administration of Ia $^g$  immunogenic but not tolerated peptide binder. Again, one would have to demonstrate that the Ia $^g$  binder in question does not act as an altered peptide ligand (APL) known to stimulate regulatory T cells in these models.

#### 5. Targeting of costimulation and adhesion molecules

The activation of autoreactive T cells specific to  $\beta$ -cell antigens involves a number of costimulation and adhesion molecules. Thus, antibodies to B7.1 or to CD40L prevent the onset of diabetes. CTLA-4-Ig, a fusion protein of CTLA-4 and IgG Fc, which inhibits the binding of CD28 to B7, also delays the onset of diabetes. A similar preventive effect has been reported for an anti-CD28 antibody, but here the mechanism of action of the antibody probably relates to an agonistic effect leading to signalling of regulatory T cells. In fact, this therapeutic approach is more generally complicated by the dual effect of some of the agents used, depending when they are administered. Thus, CTLA-4-Ig fusion protein prevents the onset of diabetes when administered late but accelerates the progression of the disease when administered early [18].

Note also that CD28 $^{-/-}$  and B7 $^{-/-}$  NOD mice show fulminant diabetes, probably because of the absence of regulatory T cells [18,19].

Diabetes has also been prevented by blocking adhesion molecules, particularly using antibodies against intercellular adhesion molecule (ICAM)-1 and LFA-1. Workers in this laboratory have recently found that administration of adenovirus-infected cells producing soluble recombinant ICAM-1 also protected NOD mice against diabetes. We have even shown that such gene therapy can reverse recently established diabetes (P Lemarchand, unpublished observations).

#### 6. Cytokine blockade

A wide array of cytokines are involved in the differentiation and activation of the various T-cell subsets contributing to diabetes pathogenesis in NOD mice. All antibodies directed at cytokines or cytokine receptors inhibiting the onset of diabetes relate to Th1 cells. Thus, the onset of diabetes is prevented by antibodies directed against IFN- $\gamma$ , IL-2 receptor (an association with low-dose cyclosporin A is required), or IL-12. Interestingly, a similar effect was obtained by blocking the cytokine receptor with a receptor/immunoglobulin fusion protein or by destroying the

S7

receptor-bearing cell with a cytokine-toxin conjugate. The preventive effect of orally administered IFN- $\alpha$  is interesting but is difficult to interpret. Also intriguing is the absence of diabetes prevention in NOD mice genetically deficient in IFN- $\gamma$ , IFN- $\gamma$  receptor, or IL-12 [20–22], a paradox probably explained by a redundancy of the genes coding for these cytokines and their receptors. Prevention of diabetes has been reported after blockade of proinflammatory cytokines, namely IL-1, IL-6, and tumour necrosis factor (TNF)- $\alpha$ . In the latter case, the effect was observed only when the neutralising antibody was administered at a very young age.

#### 7. Pharmacologically active cytokines

Many of the strategies resulting in stimulation of regulatory cells may be assumed to involve the suppressive effect of cytokines acting either systematically or locally at the islet level. The onset of diabetes may also be prevented by the direct administration of regulatory cytokines.

##### IL-4

Systemic administration of IL-4 can delay the onset of diabetes. The effect is not as dramatic as that of other procedures described here, but is nevertheless quite significant. In fact, the effect is more clear cut when the cytokine is directly delivered in the islet using either gene therapy or  $\beta$ -cell-targeted transgenesis.

##### IL-10

Findings similar to those reported for IL-4 have been reported for IL-10 after systemic administration of the recombinant cytokine. Paradoxically, however, the onset of diabetes is accelerated by intra-islet delivery of IL-10 in transgenic mice [23] or by systemic administration of an IL-10-Ig fusion protein [24], possibly due in the latter case to an unexpected Th2 polarization.

##### IL-13

A modest but significant delay in the onset of diabetes has been reportedly achieved by IL-13, another Th2 cytokine.

##### G-CSF

Granulocyte-colony-stimulating factor (G-CSF) has been used successfully to protect NOD mice from diabetes, following previous results in this laboratory showing that G-CSF could prevent systemic lupus erythematosus in (NZB  $\times$  NZW)F<sub>1</sub> mice [25]. Data collected in these various models suggest that the effect of G-CSF could involve Th2 polarisation.

##### TNF

Contrasting results have been reported for TNF. Given in the adult NOD mouse, TNF prevents the onset of diabetes [26] (an observation in keeping with the insulinitis acceleration brought about by anti-TNF antibodies). Conversely, given to newborn NOD mice, TNF accelerates disease progression [27].

##### IL-1

IL-1 has been reported to protect NOD mice from the onset of diabetes. This is a surprising observation, because IL-1 has been shown to be exquisitely toxic to  $\beta$  cells and because an IL-1 antagonist has been reported to protect against diabetes.

##### IL-12

Again depending on the protocol of administration, IL-12 may accelerate or slow down the progression of diabetes.

##### Lymphotoxin

Diabetes protection has also been reported for lymphotoxin and lymphotoxin-receptor fusion protein.

#### 8. Tolerance to soluble $\beta$ -cell autoantigens

Many efforts have been made to induce tolerance to candidate  $\beta$ -cell autoantigens. Prevention of disease (but not cure of established disease) has been obtained with insulin, glutamic acid decarboxylase, and hsp60. In the case of insulin, evidence indicated that the effect was not exclusively linked to the hormone's metabolic activity, since the disease could be prevented with insulin, metabolically inactive B chain, or inactive analogues. In the case of hsp60, the antigen is not, strictly speaking,  $\beta$ -cell-specific, but its overexpression in inflamed  $\beta$  cells leads to some  $\beta$ -cell-selective expression.

With each of these three antigens, diabetes was prevented by using various routes of administration: subcutaneous (+ adjuvant), oral, nasal, intravenous, intrathymic. Tolerance was also induced by vaccination with antigen-specific DNA, as well as by transgenic overexpression of the autoantigen.

At the level of underlying mechanisms, there is no true antigen-specific tolerance, since the downregulation of autoimmunity extends to antigens other than the tolerogen. Accumulated data show that soluble  $\beta$ -cell autoantigens induce a deviation in immunity towards Th2, with bystander suppression probably involving local release of immunosuppressive cytokines [2].

#### 9. Stimulation of regulatory T cells

The diabetogenic autoimmune response is tightly controlled by a variety of regulatory T cells. I have pointed out how the administration of soluble  $\beta$ -cell autoantigens could stimulate Th2 cells and prevent the onset of diabetes if given when the mice are young enough. Many other strategies have been used to prevent the onset of diabetes targeting non-Th2 regulatory T cells. One may assume, a priori, that most of these strategies are not  $\beta$ -cell-specific, since they use non- $\beta$ -cell-related agents. The possibility cannot be excluded that, at least in some cases, the induced regulation is  $\beta$ -cell-specific at the effector level. One may postulate that a nonspecific stimulation

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leads to the activation or boosting of  $\beta$ -cell-specific regulatory T cells, whether or not they are of the Th2 type. The strategies for stimulating regulatory T cells may be classified according to whether they make use of nondepleting anti-T-cell monoclonal antibodies, stimulation of innate immunity, or pathogens, as discussed below.

#### *Nondepleting anti-T-cell monoclonal antibodies*

Administration of anti-CD3 antibodies to NOD mice with recently manifested IDDM induces long-term remission of the disease. The effect is obtained after brief treatment (5 days) and does not require the use of the mitogenic whole autoantibody molecule (nonactivating F(ab')<sub>2</sub> fragments are tolerogenic) [28,29]. My colleagues and I have recently obtained data indicating that the effect is mediated by active tolerance involving TGF- $\beta$ -dependent CTLA<sup>+</sup>CD25<sup>+</sup> T cells (L Chatenoud, unpublished observations).

Similar, though less well documented, data have been reported for nondepleting anti-CD4 antibodies [30], in keeping with the analogous effect of the same antibodies in transplantation. [31].

#### *Stimulation of innate immunity*

NOD mice show an early deficit in NK (natural killer) T cells, both quantitatively and qualitatively (deficient IL-4 production) [32]. It was thus logical to attempt to prevent IDDM in such mice by stimulating the function of NK T cells. This was recently done by administering a selective NK-T-cell ligand, the glycolipid  $\alpha$ -galactosylceramide. Interestingly, the protection still applies in some protocols when the glycolipid is given late, and can inhibit the recurrence of disease in diabetic mice with grafts of syngeneic islets [33].

Stimulation of  $\gamma\delta$  regulatory T cells has been reported after intranasal administration of insulin [34]. It will be interesting to learn whether such T cells that protect against diabetes after nasal administration of insulin are insulin specific.

#### *Pathogens*

**Bacteria.** A whole array of bacteria have been shown to prevent the onset of diabetes in NOD mice. Mycobacteria have been extensively studied, particularly *Mycobacterium bovis* (the source of BCG vaccine) and *M. avium*. The effect is also obtained with mycobacteria extracts (in complete Freund's adjuvant). The role of regulatory T cells in protection induced by complete Freund's adjuvant or vaccination with BCG is demonstrated by the transfer of protection that is achieved when CD4 T cells from protected mice are transferred to naive mice [35]. The nature of the regulatory cells in question is open to speculation (are they Th2 cells? CD25 cells?). Other bacterial-cell extracts have also been shown to prevent the onset of diabetes in NOD mice, notably extracts of streptococcus or klebsiella.

**Viruses.** The onset of diabetes in NOD mice can be prevented by infection with various viruses, in particular lymphocoriomeningitis virus (LCMV), murine hepatitis virus (MHV), and lactate dehydrogenase virus (LDHV).

**Parasites.** Diabetes can also be prevented by deliberate administration of parasites, such as schistosomes or filariae.

#### **10. Gene therapy**

Gene therapy may be used in many ways to prevent or cure diabetes in NOD mice. Insulin gene therapy and related strategies are not discussed in this chapter.

Immune-based gene therapy has been developed along several lines. One possibility is to overexpress cytokines or cytokine receptors with the aim of reproducing the pharmacological effect of the particular molecules. Various experimental settings have been considered, including local intra-islet delivery of the cytokine (using transgenic mice or islet-specific T-cell transfection) and systemic delivery. Various vectors (viral and nonviral) have been used. IL-4, IL-4-Ig fusion protein, IL-10, IFN- $\gamma$  receptor, Ig, and TGF- $\beta$  all protected the mice from diabetes.

We recently reported that systemic delivery of soluble ICAM-1 using a recombinant adenovirus vector could also be protective and even curative in mice that had recently developed diabetes (P Lemarchand, unpublished observations).

Less expected is the protective effect of calcitonin gene therapy.

#### **11. Cell therapy**

##### *Islet transplantation*

Syngeneic islet transplantation is really a palliative procedure, not an immunotherapeutic one. However, unlike insulin therapy, it poses the problems of the prevention of disease relapse on the graft and consequently requires associated immunotherapy. Many of the procedures described above have been used to prevent such disease relapse, e.g. anti-CD3 and anti-CD4 antibodies, soluble glutamic acid decarboxylase,  $\alpha$ -galactosylceramide, and BCG vaccination. Similar immunological problems will be met with attempts to regenerate islet cells from ductal stem cells, as has been recently described. The problem is even more serious in the case of allogeneic islet transplantation, in which two problems – relapse and allograft rejection – are combined.

##### *Bone marrow transplantation*

**Allogeneic bone marrow transplantation.** Another approach consists in replacing the bone marrow T (and B) cell precursors. This is not an easy approach, because of the associated allogeneic reaction (graft versus host and host versus graft). Such alloimmune response could have a protective effect, probably through the production of

S9



immunoregulatory cytokines: this possibility is suggested by the protection afforded by induction of (usually partial) allogeneic tolerance in newborn NOD mice, which also totally protects from diabetes [36].

**Syngeneic bone marrow transplantation.** More unexpectedly, syngeneic bone marrow transplantation may also afford protection (in conjunction with IL-3), possibly by resetting immunoregulatory mechanisms that override effector ones [37].

#### *Infusion of mononuclear cells*

Prevention of diabetes has been reported after infusion of dendritic cells and CD4<sup>-/-</sup>CD8<sup>-/-</sup> thymocytes presumably enriched in NK T cells. It has also been extensively demonstrated that the onset of diabetes in NOD mice is prevented by administering mature CD4 T cells (either polyclonal, notably of the CD25 type, or monoclonal).

#### *Intrathymic islet transplantation*

Diabetes has been prevented in NOD mice upon intrathymic grafting of syngeneic or allogeneic islets, either at birth or within 4 weeks of age. The preventive effect was associated with a complete absence of insulinitis in most animals. The observations that spleen cells from tolerant islet-grafted NOD mice did not transfer diabetes into immunoincompetent hosts [38] and that cyclophosphamide did not break the tolerance in one study [39] are compatible with a preferential deletional mechanism.

### **12. Inhibition of $\beta$ -cell lesion**

Inhibition of the effector mechanisms leading to destruction of  $\beta$  cells has been attempted with limited success.

Nicotinamide has some protective effect but only at relatively high doses and early in the disease history. Nitric oxide (NO) inhibitors have also shown some effects as do antioxidants, pentoxifylline, and rolipram.

Anti-TCR antibodies and CD3 antibodies also deserve mention here. They probably act, at least in part, by inactivating effector T cells, as is suggested by virtually immediate reversal of hyperglycaemia after the first injection of such antibodies [13,28].

### **13. Miscellaneous**

#### *Immunomodulation*

Some products known to modulate immune responses (without showing a clear overall suppressor or stimulator pattern of activities) prevent the onset of diabetes in NOD mice. These include linomide, ciamexon, vanadate, vitamin D3, and D-glucan.

#### *Hormones*

Some hormones or related compounds can also prevent insulinitis and the progression of diabetes in NOD mice.

This has notably been reported for androgens, a finding in keeping with the acceleration of disease seen after castration in males and the high female/male ratio of affected mice. The onset of diabetes is also prevented by IGF I.

#### *Immunomanipulation*

Unexpectedly, immunisation against the lupus-associated idiotype 16/6 protects NOD mice from diabetes. The protective effect of natural antibodies presumably has a similar mode of action. The effect of such antibodies is interesting, but their mode of action is poorly defined.

#### *Diet*

Various diets have been shown to slow the progression of diabetes in NOD mice, notably the low-protein diets. It has been reported that casein hydrolysate formula does likewise.

#### *Other products*

A number of products listed in Table 1 that have an ill-defined action on the immune system have also been reported to prevent the onset of diabetes in NOD mice.

### **Concluding remarks**

The number and variety of therapeutic interventions capable of preventing diabetes represents an unprecedented observation in immune pathology. The number of interventions that work in mice with advanced disease, and particularly with established diabetes, is much more limited, indicating that the majority of efficacious treatments are active only at the very early stages of a chronic process progressing from insulinitis to clinical diabetes. As has been mentioned above, the only products that have been shown to arrest the destruction of  $\beta$  cells in man are those shown to act late in the natural history of the disease in NOD mice. Nevertheless, the early-acting procedures may prove useful in combination with late-acting drugs. One might envision treating patients who have recently diagnosed diabetes with the late-acting drugs, followed by administration of early-acting drugs, which would regain their activity once the immune homeostasis has been reset. Alternatively, these numerous early-acting compounds could be applied in man very early if valid prediction could identify subjects at risk of developing the disease. However, the logistic problems associated with such prediabetes trials should not be overlooked (for example, the number of subjects to be screened and enrolled and the duration of the trial). Lastly, many of the concepts and therapeutic strategies described above for IDDM could probably be extrapolated correctly to other autoimmune diseases, notably rheumatoid arthritis.

### **Glossary of terms**

BB = BioBreeding (rat); BCG = bacille Calmette-Guérin; NOD = nonobese diabetic (mouse).

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## References

- Bach JF: Insulin-dependent diabetes mellitus as an autoimmune disease. *Endocr Rev* 1994, 15:518-542. [key review]
- Bach JF, Chatenoud L: Tolerance to islet autoantigens and type 1 diabetes. *Annu Rev Immunol* 2001, 19:131-161. [key review]
- Fautren G, Papan L, Assan R, Vialella B, Karaant G, Vexiau P, Du Rostu II, Rodier M, Sirmat J, Lallemand A, Bach JF: Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* 1986, 2:119-124. [general reference]
- The Canadian-European Randomized Control Trial Group: Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes* 1988, 37:1574-1582. [general reference]
- Roz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR: Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet* 2001, 358:1749-1753. [general reference]
- Lampeter EF, Klinghammer A, Scherbaum WA, Heinze E, Haastert B, Giani G, Kolb H: The Deutsche Nicotinamide Intervention Study: an attempt to prevent type 1 diabetes. DENIS Group. *Diabetes* 1988, 37:980-984. [general reference]
- Chailous L, Lefevre H, Thivolet C, Boitard C, Lahlou N, Atlan Gepner C, Bouhanick B, Mogenet A, Nicolino M, Carat JC, Lecomte P, Marechaud R, Bougneres P, Charbonnel B, Sai P: Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Lancet* 2000, 356:545-549. [general reference]
- Pozzilli P, Pitocco D, Visalli N, Cavallo MG, Buzzetti R, Crino A, Spera S, Suraci C, Multari G, Cervoni M, Bitti MLM, Matteoli MC, Marietti G, Ferrazzoli F, Faldetta MRC, Giordano C, Sbriglia M, Sarugari E, Ghirlanda G: No effect of oral insulin on residual beta-cell function in recent-onset Type 1 diabetes (The IMDiAB VII). *Diabetologia* 2000, 43:1000-1004. [general reference]
- Allen HF, Klingensmith GJ, Jensen P, Simoes E, Hayward A, Chase HP: Effect of Bacillus Calmette-Guerin vaccination on new-onset type 1 diabetes. A randomized clinical study. *Diabetes Care* 1999, 22:1703-1707. [general reference]
- Weiner HL, Friedman A, Miller A, Khoury SJ, Al-Sabbagh A, Santos L, Sayegh M, Nussenblatt RB, Trentham DE, Hafler DA: Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu Rev Immunol* 1994, 12:809-837. [key review]
- Maki T, Ichikawa T, Blanco R, Porter J: Long-term abrogation of autoimmune diabetes in nonobese diabetic mice by immunotherapy with anti-lymphocyte serum. *Proc Natl Acad Sci USA* 1992, 89:3434-3438. [general reference]
- Wang Y, Hao L, Gill RG, Lafferty KJ: Autoimmune diabetes in NOD mouse is L3T4 T-lymphocyte dependent. *Diabetes* 1987, 36:535-538. [general reference]
- Sempe P, Bedossa P, Richard MF, Villa MC, Bach JF, Boitard C: Anti-alpha/beta T cell receptor monoclonal antibody provides an efficient therapy for autoimmune diabetes in nonobese diabetic (NOD) mice. *Eur J Immunol* 1991, 21:1163-1169. [general reference]
- Acha-Orbea H, Mitchell DJ, Timmermann L, Wraith DC, Tausch GS, Waldor MK, Zamvil SS, McDavitt HO, Steinman L: Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* 1988, 54:263-273. [general reference]
- Conrad B, Weissmahr RN, Boni J, Arcari R, Schupbach J, Mach B: A human endogenous retroviral superantigen as candidate autoimmune gene in type 1 diabetes. *Cell* 1997, 90:303-313. [general reference]
- Stattary RM, Kjer-Nielsen L, Allison J, Charlton B, Mandel TE, Miller JF: Prevention of diabetes in non-obese diabetic I-Ak transgenic mice. *Nature* 1990, 345:724-726. [general reference]
- Singer SM, Tisch R, Yang XD, McDavitt HO: An AbD transgene prevents diabetes in nonobese diabetic mice by inducing regulatory T cells. *Proc Natl Acad Sci USA* 1993, 90:9566-9570. [general reference]
- Lenschow DJ, Harold KC, Rhee L, Patel B, Koons A, Qin HY, Fuchs E, Singh B, Thompson CB, Bluestone JA: CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. *Immunity* 1998, 5:289-293. [general reference]
- Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, Bluestone JA: B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000, 12:431-440. [general reference]
- Hultgren B, Huang XJ, Dybdal N, Stewart TA: Genetic absence of gamma-interferon delays but does not prevent diabetes in NOD mice. *Diabetes* 1998, 47:812-817. [general reference]
- Serreze DV, Post CM, Chapman HD, Johnson EA, Lu BF, Rothman PB: Interferon-gamma receptor signaling is dispensable in the development of autoimmune type 1 diabetes in NOD mice. *Diabetes* 2000, 49:2007-2011. [general reference]
- Trembleau S, Penna G, Gregori S, Chapman HD, Serreze DV, Magram J, Adorini L: Pancreas-infiltrating Th1 cells and diabetes develop in IL-12-deficient nonobese diabetic mice. *J Immunol* 1999, 163:2960-2968. [general reference]
- Wogensen L, Lee MS, Sarvetnick N: Production of interleukin 10 by islet cells accelerates immune-mediated destruction of beta cells in nonobese diabetic mice. *J Exp Med* 1994, 179:1379-1384. [general reference]
- Zheng XX, Steele AW, Hancock WW, Stevens AC, Nickerson PW, Roy-Chaudhury P, Tian Y, Strom TB: A noncytolytic IL-10/Fc fusion protein prevents diabetes, blocks autoimmunity, and promotes suppressor phenomena in NOD mice. *J Immunol* 1997, 158:4507-4513. [general reference]
- Zavala F, Masson A, Hadaya K, Ezine S, Schneider E, Babin O, Bach JF: Granulocyte-colony stimulating factor treatment of lupus autoimmune disease in MRL-*lpr/lpr* mice. *J Immunol* 1999, 163:5125-5132. [general reference]
- Jacob CO, Aiso S, Michie SA, McDavitt HO, Acha-Orbea H: Prevention of diabetes in nonobese diabetic mice by tumor necrosis factor (TNF): similarities between TNF-alpha and interleukin 1. *Proc Natl Acad Sci USA* 1990, 87:968-972. [general reference]
- Yang XD, Tisch R, Singer SM, Cao ZA, Liblau RS, Schreiber RD, McDavitt HO: Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med* 1994, 180:995-1004. [general reference]
- Chatenoud L, Thervet E, Primo J, Bach JF: Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci USA* 1994, 91:123-127. [general reference]
- Chatenoud L, Primo J, Bach JF: CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J Immunol* 1997, 158:2947-2954. [general reference]
- Hutchings P, O'Reilly L, Parish NM, Waldmann H, Cooke A: The use of a non-depleting anti-CD4 monoclonal antibody to reestablish tolerance to beta cells in NOD mice. *Eur J Immunol* 1992, 22:1913-1918. [general reference]
- Waldmann H, Cobbold S: How do monoclonal antibodies induce tolerance? A role for infectious tolerance? *Annu Rev Immunol* 1998, 16:619-644. [key review]
- Gombart JM, Herbelin A, Tancrede-Behin E, Dy M, Camaud C, Bach JF: Early quantitative and functional deficiency of NK1(+)-like thymocytes in the NOD mouse. *Eur J Immunol* 1998, 28:2989-2998. [general reference]
- Sharif S, Arrasza GA, Zucker P, Mi QS, Sondhi J, Naidenko OV, Kronenberg M, Kozuka Y, Delovitch TL, Gombart JM, Leite de Moraes M, Gouarin C, Zhu R, Harnag A, Nakayama T, Taniguchi M, Lepault F, Lehen A, Bach JF, Herbelin A: Activation of natural killer T cells by  $\alpha$ -galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat Med* 2001, 7:1057-1062. [general reference]
- Harrison LC, Dempsey-Collier M, Kramer DR, Takahashi K: Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. *J Exp Med* 1996, 184:2167-2174. [general reference]
- Qin HY, Sadefain MW, Hichon C, Lauzon J, Singh B: Complete Freund's adjuvant-induced T cells prevent the development and adoptive transfer of diabetes in nonobese diabetic mice. *J Immunol* 1993, 150:2072-2080. [general reference]

S11

36. Bendelac A, Boitard C, Bach JF, Carnaud C: Neonatal induction of allogeneic tolerance prevents T cell-mediated autoimmunity in NOD mice. *Eur J Immunol* 1989, 19:811-816. [general reference]
37. Ito A, Aoyanagi N, Maki T: Regulation of autoimmune diabetes by interleukin 3-dependent bone marrow-derived cells in NOD mice. *J Autoimmun* 1997, 10:331-338. [general reference]
38. Gerling IC, Serrero DV, Christianson SW, Leiter EH: Intrathymic islet cell transplantation reduces beta-cell autoimmunity and prevents diabetes in NOD/Lt mice. *Diabetes* 1992, 41:1672-1676. [general reference]
39. Charlton B, Taylor-Edwards C, Tisch R, Fathman CG: Prevention of diabetes and insulinitis by neonatal intrathymic islet administration in NOD mice. *J Autoimmun* 1994, 7:549-560. [general reference]
40. Shizuru JA, Taylor-Edwards C, Banks BA, Gregory AK, Fathman CG: Immunotherapy of the nonobese diabetic mouse: treatment with an antibody to T-helper lymphocytes. *Science* 1988, 240:659-662. [general reference]
41. Hutchings PR, Simpson E, O'Reilly LA, Lund T, Waldmann H, Cooke A: The involvement of Ly2+ T cells in beta cell destruction. *J Autoimmun* 1990, 3 (Suppl 1):101-109. [general reference]
42. Weiss L, Slavin S, Reich S, Cohen P, Shuster S, Stern R, Kaganovsky E, Okon E, Rubinstein AM, Naor D: Induction of resistance to diabetes in non-obese diabetic mice by targeting CD44 with a specific monoclonal antibody. *Proc Natl Acad Sci USA* 2000, 97:285-290. [general reference]
43. Sempé P, Ezine S, Marvel J, Bedossa P, Richard MF, Bach JF, Boitard C: Role of CD4+CD45RA+ T cells in the development of autoimmune diabetes in the non-obese diabetic (NOD) mouse. *Int Immunol* 1993, 5:479-489. [general reference]
44. Abu-Hadid MM, Lazarovits AJ, Madrenas J: Prevention of diabetes mellitus in the non-obese diabetic mouse strain with monoclonal antibodies against the CD45RB molecule. *Autoimmunity* 2000, 32:72-78. [general reference]
45. Harada M, Makino S: Suppression of overt diabetes in NOD mice by anti-thymocyte serum or anti-Thy 1, 2 antibody. *Jikken Dobutsu* 1988, 4:501-504. [general reference]
46. Ogawa M, Maruyama T, Hasegawa T, Kanaya T, Kobayashi F, Tochino Y, Uda H: The inhibitory effect of neonatal thymectomy on the incidence of insulinitis in non-obese diabetic (NOD) mice. *Biomed Res* 1985, 6:103-105. [general reference]
47. Johnson GG, Mikulowska A, Butcher EC, McEvoy LM, Michie SA: Anti-CD43 monoclonal antibody L11 blocks migration of T cells to inflamed pancreatic islets and prevents development of diabetes in nonobese diabetic mice. *J Immunol* 1999, 163:5678-5685. [general reference]
48. Takamoto K, Yokono K, Amano K, Nagata M, Yagi N, Tominaga Y, Moriyama H, Miki M, Okamoto N, Yoneda R: Administration of monoclonal antibodies against vascular cell adhesion molecule-1/very late antigen-4 abrogates predisposing autoimmune diabetes in NOD mice. *Cell Immunol* 1995, 165:193-201. [general reference]
49. Yang XD, Karin N, Tisch R, Steinman L, McDevitt HO: Inhibition of insulitis and prevention of diabetes in nonobese diabetic mice by blocking L-selectin and very late antigen 4 adhesion receptors. *Proc Natl Acad Sci USA* 1993, 90:10494-10498. [general reference]
50. Jakubowski A, Ehrenfels BN, Pepinsky RB, Burdick LC: Vascular cell adhesion molecule-1g fusion protein selectively targets activated alpha 4-integrin receptors in vivo. Inhibition of autoimmune diabetes in an adoptive transfer model in nonobese diabetic mice. *J Immunol* 1995, 155:938-946. [general reference]
51. Mori Y, Suko M, Okudaira H, Matsuba I, Tsuruoka A, Sasaki A, Yokoyama H, Tanase T, Shida T, Nishimura M, Terada E, Ikeda Y: Preventive effects of cyclosporin on diabetes in NOD mice. *Diabetologia* 1988, 28:244-247. [general reference]
52. Miyagawa J, Yamamoto K, Hanafusa T, Itoh N, Nakagawa C, Otsuka A, Katsura H, Yamagata K, Miyazaki A, Kono N, Tanai S: Preventive effect of a new immunosuppressant FK-506 on insulitis and diabetes in non-obese diabetic mice. *Diabetologia* 1990, 33:503-505. [general reference]
53. Calafiori R, Basta G, Falorni A, Pietropaolo M, Picchio ML, Calcinario F, Brunetti P: Preventive effects of azathioprine (AZA) on the onset of diabetes mellitus in NOD mice. *J Endocrinol Invest* 1993, 16:869-873. [general reference]
54. Baeder WL, Sredy J, Sehgal SN, Chang JY, Adams LM: Rapamycin prevents the onset of insulin-dependent diabetes mellitus (IDDM) in NOD mice. *Clin Exp Immunol* 1992, 89:174-178. [general reference]
55. Nicoletti F, Borghi MO, Meroni PL, Barcellini W, Fain C, Di Marco R, Mella R, Sidielenner IU, Druso C, Magro G, Grassi S: Prevention of cyclophosphamide-induced diabetes in the NOD/WEHI mouse with deoxyspergualin. *Clin Exp Immunol* 1993, 91:232-238. [general reference]
56. Takahashi M, Kojima S, Yamaoka K, Niki E: Prevention of type 1 diabetes by low-dose gamma irradiation in NOD mice. *Radiat Res* 2000, 154:680-685. [general reference]
57. Bacallj A, Charlton B, Mandel TE: Prevention of cyclophosphamide-induced diabetes by anti-V beta 8 T-lymphocyte-receptor monoclonal antibody therapy in NOD/Wehi mice. *Diabetes* 1989, 38:1492-1495. [general reference]
58. Gerson CL, Hussain MJ, Vergani D, Peakman M: Lymphocyte vaccination protects prediabetic non-obese diabetic mice from developing diabetes mellitus. *Diabetologia* 1997, 40:1388-1393. [general reference]
59. Smeardon RA, Peakman M, Hussain MJ, Vergani D: Lymphocyte vaccination prevents spontaneous diabetes in the non-obese diabetic mouse. *Immunology* 1993, 80:498-501. [general reference]
60. Formby B, Shao T: T cell vaccination against autoimmune diabetes in nonobese diabetic mice. *Ann Clin Lab Sci* 1993, 23:137-147. [general reference]
61. Feil-Harini M, Frantz MO, Morel PA: Prevention of diabetes in the NOD mouse by a Th1 clone specific for a hsp60 peptide. *J Autoimmun* 2000, 14:133-142. [general reference]
62. Vaysburd M, Lock C, McDevitt H: Prevention of insulin-dependent diabetes mellitus in nonobese diabetic mice by immunogenic but not by tolerated peptides. *J Exp Med* 1995, 182:897-902. [general reference]
63. Taki T, Nagata M, Ogawa W, Hatamori N, Hayakawa M, Hari J, Shii K, Baba S, Yokono K: Prevention of cyclophosphamide-induced and spontaneous diabetes in NOD/Shi/Kbs mice by anti-MHC class I Kd monoclonal antibody. *Diabetes* 1991, 40:1203-1209. [general reference]
64. Boitard C, Bendelac A, Richard MF, Carnaud C, Bach JF: Prevention of diabetes in nonobese diabetic mice by anti-I-A monoclonal antibodies: transfer of protection by splenic T cells. *Proc Natl Acad Sci USA* 1988, 85:9719-9723. [general reference]
65. Miyazaki T, Matsuda Y, Toyonaga T, Miyazaki J, Yazaki Y, Yamamura K: Prevention of autoimmune insulitis in nonobese diabetic mice by expression of major histocompatibility complex class I Id molecules. *Proc Natl Acad Sci USA* 1992, 89:9519-9523. [general reference]
66. Singer SM, Tisch R, Yang XD, Sytwu HK, Liblau R, McDevitt HO: Prevention of diabetes in NOD mice by a mutated I-A transgene. *Diabetes* 1998, 47:1570-1577. [general reference]
67. Nishimoto H, Kikuchi H, Yamamura K, Kishimoto T: Prevention of autoimmune insulitis by expression of I-E molecules in NOD mice. *Nature* 1987, 328:432-434. [general reference]
68. Amazea GA, Cameron MJ, Jaramillo A, Gill BM, Hardy D, Laupland KB, Rapoport MJ, Zucker P, Chakrabarti S, Chensue SW, Qin HY, Singh B, Delovitch TL: Neonatal activation of CD28 signalling overcomes T cell anergy and prevents autoimmune diabetes by an IL-4-dependent mechanism. *J Clin Invest* 1997, 100:2243-2253. [general reference]
69. Lenaschow DJ, Ho SC, Saitta H, Rhee L, Gray G, Nabavi N, Herold KC, Bluestone JA: Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 1995, 181:1145-1155. [general reference]
70. Balasa B, Krahl T, Patstone G, Lee J, Tisch R, McDevitt HO, Sarvetnick N: CD40 ligand-CD40 interactions are necessary for the initiation of insulitis and diabetes in nonobese diabetic mice. *J Immunol* 1997, 159:4620-4627. [general reference]
71. Hasegawa Y, Yokono K, Taki T, Amano K, Tominaga Y, Yoneda R, Yagi N, Maeda S, Yagita H, Okumura K, Kasuga M: Prevention of autoimmune insulin-dependent diabetes in non-obese diabetic mice by anti-LFA-1 and anti-ICAM-1 mAb. *Int Immunol* 1994, 6:831-838. [general reference]
72. Martin S, Haidenthal E, Schulte B, Rothe H, Kolb H: Soluble forms of intercellular adhesion molecule-1 inhibit insulitis and

Available online <http://arthritis-research.com/content/4/S3/S003>

- onset of autoimmune diabetes. *Diabetologia* 1998, 41:1298-1303. [general reference]
73. Hutchings P, Rosen H, O'Reilly L, Simpson E, Gordon S, Cooke A: Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature* 1990, 340:639-642. [general reference]
  74. Debray-Sachs M, Camaud C, Borlard C, Cohen H, Gresser I, Bodonca P, Bach JF: Prevention of diabetes in NOD mice treated with antibody to murine IFN gamma. *J Autoimmun* 1991, 4:237-248. [general reference]
  75. Campbell IL, Kay TW, Oxbrow L, Harrison LC: Essential role for interferon-gamma and interleukin-6 in autoimmune insulin-dependent diabetes in NOD/Wehi mice. *J Clin Invest* 1991, 87:739-742. [general reference]
  76. Prudhomme GJ, Chang Y: Prevention of autoimmune diabetes by intramuscular gene therapy with a nonviral vector encoding an interferon-gamma receptor/IgG1 fusion protein. *Gene Ther* 1999, 6:771-777. [general reference]
  77. Kelley VE, Gaulton GN, Hattori M, Ikegami H, Eisenbarth G, Strom TB: Anti-interleukin 2 receptor antibody suppresses murine diabetic insulinitis and lupus nephritis. *J Immunol* 1988, 140:59-61. [general reference]
  78. Zheng XX, Steele AW, Hancock WW, Kawamoto K, Li XC, Nickerson PW, Li Y, Tian Y, Strom TB: IL-2 receptor-targeted cytolytic IL-2/Fc fusion protein treatment blocks diabetogenic autoimmunity in nonobese diabetic mice. *J Immunol* 1999, 163:4041-4048. [general reference]
  79. Pacheco-Silva A, Bastos MG, Muggia RA, Pankewycz O, Nichols J, Murphy JR, Strom TB, Rubin-Kelley VE: Interleukin 2 receptor targeted fusion toxin (DAB486-IL-2) treatment blocks diabetogenic autoimmunity in non-obese diabetic mice. *Eur J Immunol* 1992, 22:697-702. [general reference]
  80. Fujihira K, Nagata M, Moriyama H, Yasuda H, Arisawa K, Nakayama M, Maeda S, Kasuga M, Okumura K, Yagita H, Yokono K: Suppression and acceleration of autoimmune diabetes by neutralization of endogenous interleukin-12 in NOD mice. *Diabetes* 2000, 49:1998-2006. [general reference]
  81. Rothe H, O'Hara RM Jr, Martin S, Kolb H: Suppression of cyclophosphamide induced diabetes development and pancreatic Th1 reactivity in NOD mice treated with the interleukin (IL)-12 antagonist IL-12(p40)2. *Diabetologia* 1997, 40:641-646. [general reference]
  82. Brod SA, Malone M, Darcas S, Papolla M, Nelson L: Ingested interferon alpha suppresses type 1 diabetes in non-obese diabetic mice. *Diabetologia* 1988, 41:1227-1232. [general reference]
  83. Caillaud C, Diu-Hercend A, Ruith E, Westwood R, Camaud C: Treatment with neutralizing antibodies specific for IL-1beta prevents cyclophosphamide-induced diabetes in nonobese diabetic mice. *Diabetes* 1997, 46:937-940. [general reference]
  84. Sandberg JO, Etzirik DL, Sandler S: IL-1 receptor antagonist inhibits recurrence of disease after syngeneic pancreatic islet transplantation to spontaneously diabetic non-obese diabetic (NOD) mice. *Clin Exp Immunol* 1997, 108:314-317. [general reference]
  85. Wu Q, Salomon B, Chen M, Wang Y, Hoffman LM, Bluestone JA, Fu YX: Reversal of spontaneous autoimmune insulinitis in nonobese diabetic mice by soluble lymphotoxin receptor. *J Exp Med* 2001, 193:1327-1332. [general reference]
  86. Rapoport MJ, Iaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, Oyopick P, Danska JS, Delovitch TL: Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J Exp Med* 1993, 178:87-99. [general reference]
  87. Rabinovitch A, Suarez-Pinzon WL, Sorensen O, Bleackley RC, Power RF, Rajotte RV: Combined therapy with interleukin-4 and interleukin-10 inhibits autoimmune diabetes recurrence in syngeneic islet-transplanted nonobese diabetic mice. Analysis of cytokine mRNA expression in the graft. *Transplantation* 1995, 60:368-374. [general reference]
  88. Pennline KJ, Roque-Gaffney E, Monahan M: Recombinant human IL-10 prevents the onset of diabetes in the nonobese diabetic mouse. *Clin Immunol Immunopathol* 1994, 71:169-175. [general reference]
  89. Zaccane P, Phillips J, Conget I, Gomis R, Haskins K, Minty A, Bendtzen K, Cooke A, Nicoletti F: Interleukin-13 prevents autoimmune diabetes in NOD mice. *Diabetes* 1999, 48:1522-1528. [general reference]
  90. Kim JY, Cho SH, Kim YW, Jang EC, Park SY, Kim EJ, Lee SK: Effects of BCG, lymphotoxin and bee venom on insulinitis and development of IDDM in non-obese diabetic mice. *J Kor Med Sci* 1999, 14:648-652. [general reference]
  91. Nicoletti F, Zaccane P, Conget I, Gomis R, Moller C, Maroni PL, Bendtzen K, Trepicchio W, Sandler S: Early prophylaxis with recombinant human interleukin-11 prevents spontaneous diabetes in NOD mice. *Diabetes* 1999, 48:2333-2339. [general reference]
  92. Fomby B, Jacobs C, Dubuc P, Shao T: Exogenous administration of IL-1 alpha inhibits active and adoptive transfer autoimmune diabetes in NOD mice. *Autoimmunity* 1992, 12:21-27. [general reference]
  93. Zhang ZJ, Davidson L, Eisenbarth G, Weiner HL: Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci USA* 1991, 88:10262-10266. [general reference]
  94. Slavin AJ, Maron R, Weiner HL: Mucosal administration of IL-10 enhances oral tolerance in autoimmune encephalomyelitis and diabetes. *Int Immunol* 2001, 13:825-833. [general reference]
  95. Daniel D, Wegmann DR: Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9-23). *Proc Natl Acad Sci USA* 1996, 93:956-960. [general reference]
  96. Muir A, Peck A, Clare-Salzer M, Song YH, Cornelius J, Luchetta R, Krischer J, MacLaren N: Insulin immunization of nonobese diabetic mice induces a protective insulinitis characterized by diminished intrasitot interferon-gamma transcription. *J Clin Invest* 1995, 95:628-634. [general reference]
  97. Karounos DG, Bryson JS, Cohen DA: Metabolically inactive insulin analog prevents type 1 diabetes in prediabetic NOD mice. *J Clin Invest* 1997, 100:1344-1348. [general reference]
  98. Urbanek-Ruiz I, Ruiz PJ, Paragas V, Garren H, Steinman L, Fathman CG: Immunization with DNA encoding an immunodominant peptide of insulin prevents diabetes in NOD mice. *Clin Immunol* 2001, 100:164-171. [general reference]
  99. French MB, Allison J, Cram DS, Thomas HE, Dempsey-Collier M, Silva A, Georgiou HM, Kay TW, Harrison LC, Lew AM: Transgenic expression of mouse proinsulin II prevents diabetes in nonobese diabetic mice. *Diabetes* 1997, 46:34-39. [general reference]
  100. Bergerot I, Ploix C, Petersen J, Moulin V, Rask C, Fabien N, Lindblad M, Mayer A, Czerniksky C, Holmgren J, Thivole C: A cholera toxinoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc Natl Acad Sci USA* 1997, 94:4610-4614. [general reference]
  101. Ramiya VK, Shang XZ, Wasserfall CH, MacLaren NK: Effect of oral and intravenous insulin and glutamic acid decarboxylase in NOD mice. *Autoimmunity* 1997, 26:189-191. [general reference]
  102. Tian J, Atkinson MA, Clare Salzer M, Herschenfeld A, Forsthuber T, Lehmann PV, Kaufman DL: Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J Exp Med* 1996, 183:1561-1567. [general reference]
  103. Kaufman DL, Clare-Salzer M, Tian J, Forsthuber T, Ting GSP, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV: Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 1993, 366:69-72. [general reference]
  104. Tisch R, Yang XD, Singer SM, Ublau RS, Fugger L, McDavitt HO: Immune response to glutamic acid decarboxylase correlates with insulinitis in non-obese diabetic mice. *Nature* 1993, 366:72-75. [general reference]
  105. Filippova M, Liu J, Escher A: Effects of plasmid DNA injection on cyclophosphamide-accelerated diabetes in NOD mice. *DNA Cell Biol* 2001, 20:175-81. [general reference]
  106. Manard V, Jacobs M, Jun HS, Yoon JW, Kim SW: Anti-GAD monoclonal antibody delays the onset of diabetes mellitus in NOD mice. *Pharmaceut Res* 1999, 16:1059-1066. [general reference]
  107. Elias D, Markovits D, Reshef T, Van Der Zee R, Cohen IR: Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci USA* 1990, 87:1576-1580. [general reference]
  108. Elias D, Meilin A, Ablamunits V, Birk OS, Cami P, Konen-Waisman S, Cohen IR: Hsp60 peptide therapy of NOD mouse diabetes induces a Th2 cytokine burst and downregulates

- autoimmunity to various beta-cell antigens. *Diabetes* 1997, 46:758-764. [general reference]
109. Elias D, Cohen IR: Peptide therapy for diabetes in NOD mice. *Lancet* 1994, 343:704-708. [general reference]
  110. Birk OS, Douek DC, Elias D, Takacs K, Dewchand H, Gur SL, Walker MD, Van Der Zee R, Cohen IR, Altmann DM: A role of hsp60 in autoimmune diabetes: analysis in a transgenic model. *Proc Natl Acad Sci USA* 1996, 93:1032-1037. [general reference]
  111. Reddy S, Stefanovic N, Karanam M: Prevention of autoimmune diabetes by oral administration of syngeneic pancreatic extract to young NOD mice. *Pancreas* 2000, 20:55-60. [general reference]
  112. Yagi H, Matsumoto M, Kishimoto Y, Makino S, Harada M: Possible mechanism of the preventive effect of BCG against diabetes mellitus in NOD mouse. II. Suppression of pathogenesis by macrophage transfer from BCG-vaccinated mice. *Cell Immunol* 1991, 138:142-149. [general reference]
  113. Bras A, Aguas AP: Diabetes-prone NOD mice are resistant to *Mycobacterium avium* and the infection prevents autoimmune disease. *Immunology* 1996, 89:20-25. [general reference]
  114. Sadelain MW, Qin HY, Lauzon J, Singh B: Prevention of type I diabetes in NOD mice by adjuvant immunotherapy. *Diabetes* 1990, 39:583-589. [general reference]
  115. Matsuzaki T, Nagata Y, Kado S, Uchida K, Kato I, Hashimoto S, Yokura T: Prevention of onset in an insulin-dependent diabetes mellitus model, NOD mice, by oral feeding of *Lactobacillus casei*. *APMIS* 1997, 105:643-649. [general reference]
  116. Toyota T, Satoh K, Oya K, Shintani S, Okano T: Streptococcal preparation (OK-432) inhibits development of type I diabetes in NOD mice. *Diabetes* 1986, 35:486-499. [general reference]
  117. Sai P, Rivereau AS: Prevention of diabetes in the nonobese diabetic mouse by oral immunological treatments. Comparative efficiency of human insulin and two bacterial antigens, lipopolysaccharide from *Escherichia coli* and glycoprotein extract from *Klebsiella pneumoniae*. *Diabetes Metab* 1996, 22: 341-348. [general reference]
  118. Hartmann B, Bellmann K, Ghies I, Kleemann R, Kolb H: Oral insulin for diabetes prevention in NOD mice: potentiation by enhancing Th2 cytokine expression in the gut through bacterial adjuvant. *Diabetologia* 1997, 40:902-909. [general reference]
  119. Wilberz S, Parke HJ, Dagnase-Hansen F, Herberg L: Persistent MHV (mouse hepatitis virus) infection reduces the incidence of diabetes mellitus in non-obese diabetic mice. *Diabetologia* 1991, 34:2-5. [general reference]
  120. Takei I, Asaba Y, Kasatani T, Maruyama T, Watanabe K, Yanagawa T, Saruta T, Ishii T: Suppression of development of diabetes in NOD mice by lactate dehydrogenase virus infection. *J Autoimmun* 1992, 5:665-673. [general reference]
  121. Oldstone MB: Viruses as therapeutic agents. I. Treatment of nonobese insulin-dependent diabetes mice with virus prevents insulin-dependent diabetes mellitus while maintaining general immune competence. *J Exp Med* 1990, 171:2077-2089. [general reference]
  122. Imai S, Tezuka H, Fujita K: A factor of inducing IgE from a filarial parasite prevents insulin-dependent diabetes mellitus in nonobese diabetic mice. *Biochem Biophys Res Comm* 2001, 286:1051-1058. [general reference]
  123. Cooke A, Tonks P, Jones FM, O'Shea H, Hutchings P, Fulford AJ, Dunne DW: Infection with *Schistosoma mansoni* prevents insulin dependent diabetes mellitus in non-obese diabetic mice. *Parasite Immunol* 1999, 21:169-176. [general reference]
  124. Hong S, Wilson MT, Serizawa I, Wu L, Singh N, Naidenko OV, Miura T, Haba T, Scherer DC, Wei J, Kronenberg M, Kozuka Y, Van Kaer L: The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat Med* 2001, 7:1052-1056. [general reference]
  125. Kawamura T, Nagata M, Utsugi T, Yoon JW: Prevention of autoimmune type I diabetes by CD4+ suppressor T cells in superantigen-treated non-obese diabetic mice. *J Immunol* 1993, 151:4362-4370. [general reference]
  126. Tisch R, Wang B, Weaver DJ, Liu S, Bui T, Arthos J, Serreze DV: Antigen-specific mediated suppression of beta cell autoimmunity by plasmid DNA vaccination. *J Immunol* 2001, 166: 2122-2132. [general reference]
  127. Yamamoto AM, Chernajovsky Y, Lepault F, Podhajcer O, Feldman M, Bach JF, Chatenoud L: The activity of immunoregulatory T cells mediating active tolerance is potentiated in nonobese diabetic mice by an IL-4-based retroviral gene therapy. *J Immunol* 2001, 166:4973-4980. [general reference]
  128. Cameron MJ, Strathdee CA, Holmes KD, Aronza GA, Dekaban GA, Delovitch TL: Biotin-mediated interleukin 4 gene transfer prevents the onset of type 1 diabetes. *Hum Gene Ther* 2000, 11:1647-1656. [general reference]
  129. Cameron MJ, Aronza GA, Waldhauser L, Gaudin J, Delovitch TL: Immunotherapy of spontaneous type 1 diabetes in nonobese diabetic mice by systemic interleukin-4 treatment employing adenovirus vector-mediated gene transfer. *Gene Ther* 2000, 7: 1840-1846. [general reference]
  130. Chang YG, Prudhomme GJ: Intramuscular administration of expression plasmids encoding interferon-gamma receptor/ IgG1 or IL-4/IgG1 chimeric proteins protects from autoimmunity. *J Gene Med* 1999, 1:415-423. [general reference]
  131. Moritani M, Yoshimoto K, Ii S, Kondo M, Iwahana H, Yamaoka T, Sano T, Nakano N, Kikuchi H, Nakura M: Prevention of adoptively transferred diabetes in nonobese diabetic mice with IL-10-transduced islet-specific Th1 lymphocytes - A gene therapy model for autoimmune diabetes. *J Clin Invest* 1996, 98:1851-1859. [general reference]
  132. Kawamoto S, Nitta Y, Tashiro F, Nakano A, Yamato E, Tahara H, Tabayashi K, Miyazaki J: Suppression of Th1 cell activation and prevention of autoimmune diabetes in NOD mice by local expression of viral IL-10. *Int Immunol* 2001, 13:685-694. [general reference]
  133. Koh JJ, Ko KS, Lee M, Han S, Park JS, Kim SW: Degradable polymeric carrier for the delivery of IL-10 plasmid DNA to prevent autoimmune insulinitis of NOD mice. *Gene Ther* 2000, 7:2099-2104. [general reference]
  134. Piccirillo CA, Chang YG, Prudhomme GJ: TGF-beta 1 somatic gene therapy prevents autoimmune disease in nonobese diabetic mice. *J Immunol* 1998, 161:3950-3956. [general reference]
  135. Khachatryan A, Guerder S, Palluault F, Cote G, Solimena M, Valentijn K, Millet I, Flavell RA, Vignery A: Targeted expression of the neuropeptide calcitonin gene-related peptide to beta cells prevents diabetes in NOD mice. *J Immunol* 1997, 158:1409-1416. [general reference]
  136. Mottram PL, Murray-Segal LJ, Han W, Maguire J, Stein-Oakley A, Mandel TE: Long-term survival of segmental pancreas isografts in NOD/Lt mice treated with anti-CD4 and anti-CD8 monoclonal antibodies. *Diabetes* 1998, 47:1399-1405. [general reference]
  137. Yasumizu R, Sugiyama K, Iwai H, Inaba M, Makino S, Ida T, Imura H, Hamashima Y, Good RA, Ikehara S: Treatment of type 1 diabetes mellitus in non-obese diabetic mice by transplantation of allogeneic bone marrow and pancreatic tissue. *Proc Natl Acad Sci USA* 1987, 84:8555-8557. [general reference]
  138. Mathieu C, Castels K, Bouillon R, Waer M: Protection against autoimmune diabetes in mixed bone marrow chimeras: mechanisms involved. *J Immunol* 1997, 158:1453-1457. [general reference]
  139. Felli-Hariri M, Dong X, Alber SM, Watkins SC, Salter RD, Morel PA: Immunotherapy of NOD mice with bone marrow-derived dendritic cells. *Diabetes* 1999, 48:2300-2308. [general reference]
  140. Clare-Salzler MJ, Brooks J, Chai A, Van Herle K, Anderson C: Prevention of diabetes in nonobese diabetic mice by dendritic cell transfer. *J Clin Invest* 1992, 90:741-748. [general reference]
  141. Hammond KJ, Poulton LD, Palmisano LJ, Silveira PA, Godfrey DI, Baxter AG: Alpha/beta-T cell receptor (TCR)(+)/CD4(-)/CD8(-) (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J Exp Med* 1998, 187:1047-1058. [general reference]
  142. Boitard C, Yasunami R, Dardenne M, Bach JF: T cell-mediated inhibition of the transfer of autoimmune diabetes in NOD mice. *J Exp Med* 1989, 169:1889-1890. [general reference]
  143. Chosich N, Harrison LC: Suppression of diabetes mellitus in the non-obese diabetic (NOD) mouse by an autoreactive (anti-I-Ag7) islet-derived CD4+ T-cell line. *Diabetologia* 1993, 36:716-721. [general reference]
  144. Georgiou HM, Constantinou D, Mandel TE: Prevention of autoimmunity in nonobese diabetic (NOD) mice by neonatal transfer of allogeneic thymic macrophages. *Autoimmunity* 1995, 21:89-97. [general reference]

Available online <http://arthritis-research.com/content/4/S3/S003>

145. Yamada K, Nonaka K, Hanafusa T, Miyazaki A, Toyoshima H, Tarui S: Preventive and therapeutic effects of large-dose nicotinamide injections on diabetes associated with insulinitis. An observation in nonobese diabetic (NOD) mice. *Diabetes* 1982, 31:749-753. [general reference]
146. Hayward AR, Shriber M, Sokol R: Vitamin E supplementation reduces the incidence of diabetes but not insulinitis in NOD mice. *J Lab Clin Med* 1992, 119:503-507. [general reference]
147. Heineke EW, Johnson MB, Dillberger JE, Robinson KM: Antioxidant MDL 29,311 prevents diabetes in nonobese diabetic and multiple low-dose STZ-injected mice. *Diabetes* 1993, 42:1721-1730. [general reference]
148. Rabinovitch A, Suarez WL, Power RF: Combination therapy with an antioxidant and a corticosteroid prevents autoimmune diabetes in NOD mice. *Life Sci* 1992, 51:1937-1943. [general reference]
149. Corbett JA, Mikhael A, Shimizu J, Frederick K, Misko TP, McDaniel ML, Kanagawa O, Unanue ER: Nitric oxide production in islets from nonobese diabetic mice: aminoguanidine-sensitive and -resistant stages in the immunological diabetic process. *Proc Natl Acad Sci USA* 1993, 90:8992-8995. [general reference]
150. Liang L, Beshay E, Prud'Homme GJ: The phosphodiesterase inhibitors pentoxifylline and rolipram prevent diabetes in NOD mice. *Diabetes* 1998, 47:570-575. [general reference]
151. Gross DJ, Sidi H, Weiss L, Kalland T, Rosenmann E, Slavin S: Prevention of diabetes mellitus in non-obese diabetic mice by linomide, a novel immunomodulating drug. *Diabetologia* 1994, 37:1195-1201. [general reference]
152. Kino K, Mizumoto K, Sone T, Yamaji T, Watanabe J, Yamashita A, Yamaoka K, Shimizu K, Ko K, Tsunoo H: An immunomodulating protein, Ling Zhi-8 (LZ-8) prevents insulinitis in non-obese diabetic mice. *Diabetologia* 1990, 33:713-718. [general reference]
153. Kida K, Kaino Y, Ito T, Hirai H: Controversies on the prevention of insulin-dependent diabetes mellitus by immunomodulation: lessons from NOD mice treated with beta-1,6;1,3-D-glucan and rhIGF-I. *J Pediatr Endocrinol Metab* 1998, 11 (Suppl 2):327-333. [general reference]
154. Panerai AE, Nicoletti F, Sacedote P, Arvidsson L, Conget I, Gomis R, Bartorelli A, Sandler S: MFP14, a multifunctional emerging protein with immunomodulatory properties, prevents spontaneous and recurrent autoimmune diabetes in NOD mice. *Diabetologia* 2001, 44:839-47. [general reference]
155. Krug J, Lampeter EF, Williams AJ, Procaccini E, Cartledge C, Signore A, Beales PE, Pozzilli P: Immunotherapy with clomexon in the non obese diabetic (NOD) mouse. *Hormone Metab Res* 1992, 24:1-4. [general reference]
156. Burkart V, Kim Y, Kauer M, Kolb H: Induction of tolerance in macrophages by cholera toxin B chain. *Pathobiology* 1999, 67: 314-317. [general reference]
157. Sobel DO, Yankelovich B, Goyal D, Nelson D, Mazumder A: The B-subunit of cholera toxin induces immunoregulatory cells and prevents diabetes in the NOD mouse. *Diabetes* 1998, 47: 186-191. [general reference]
158. Meyerovitch J, Waner T, Sack J, Kopolovic J, Shemer J: Attempt to prevent the development of diabetes in non-obese diabetic mice by oral vanadate administration. *Israel Med Assoc J* 2000, 2:211-214. [general reference]
159. Castells KM, Mathieu C, Waer M, Valckx D, Overbergh L, Laureys JM, Bouillon R: Prevention of type 1 diabetes in nonobese diabetic mice by late intervention with nonhypercalcemic analogs of 1,25-dihydroxyvitamin D-3 in combination with a short induction course of cyclosporin A. *Endocrinology* 1998, 139:95-102. [general reference]
160. Fox HS: Androgen treatment prevents diabetes in nonobese diabetic mice. *J Exp Med* 1992, 175:1409-1412. [general reference]
161. Andersson A, Forsgren S, Soderstrom A, Holmberg O: Monoclonal, natural antibodies prevent development of diabetes in the non-obese diabetic (NOD) mouse. *J Autoimmun* 1991, 4: 733-742. [general reference]
162. Forsgren S, Andersson A, Hillom V, Soderstrom A, Holmberg O: Immunoglobulin-mediated prevention of autoimmune diabetes in the non-obese diabetic (NOD) mouse. *Scand J Immunol* 1991, 34:445-451. [general reference]
163. Krause I, Tomer Y, Elias D, Blank M, Gilburd B, Cohen IR, Shoenfeld Y: Inhibition of diabetes in NOD mice by idiotype induction of SLE. *J Autoimmun* 1999, 13:49-55. [general reference]
164. Tian J, Zekzer D, Hansson I, Lu Y, Olsson A, Kaufman DL: Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol* 2001, 167:1081-1089. [general reference]
165. Iguchi M, Inagawa H, Nishizawa T, Okutomi T, Morikawa A, Soma GI, Mizuno D: Homeostasis as regulated by activated macrophage. V. Suppression of diabetes mellitus in non-obese diabetic mice by LPSw (a lipopolysaccharide from wheat flour). *Chem Pharmaceut Bull* 1992, 40:1004-1006. [general reference]
166. Hermitte L, Allan-Gepner C, Payan MJ, Mehlelo M, Vialettes B: Dietary protection against diabetes in NOD mice: lack of a major change in the immune system. *Diabetes Metab* 1995, 21: 261-268. [general reference]
167. Hoorfar J, Buschard K, Dagnaes Hansen F: Prophylactic nutritional modification of the incidence of diabetes in autoimmune non-obese diabetic (NOD) mice. *Br J Nutr* 1993, 69: 597-607. [general reference]
168. Buschard K, Hanagers K, Fradman P, Reich EP: Treatment with sulfatide or its precursor, galactosylceramide, prevents diabetes in NOD mice. *Autoimmunity* 2001, 34:9-17. [general reference]
169. Kobayashi T, Song QH, Hong T, Kitamura H, Cyong JC: Preventive effect of Ninjin-to (Ren-Shen-Tang), a Kampo (Japanese traditional) formulation, on spontaneous autoimmune diabetes in non-obese diabetic (NOD) mice. *Microbiol Immunol* 2000, 44:299-305. [general reference]
170. Charlton B, Bacelj A, Mandal TE: Administration of silica particles or anti-Lyt2 antibody prevents beta-cell destruction in NOD mice given cyclophosphamide. *Diabetes* 1988, 37:930-935. [general reference]
171. Wilberz S, Herberg L, Renold AE: Gangliosides in vivo reduce diabetes incidence in non-obese diabetic mice. *Diabetologia* 1988, 31:855-857. [general reference]
172. Maruyama T, Watanabe K, Takei I, Kasuga A, Shimada A, Yanagawa T, Kasatani T, Suzuki Y, Kataoka K, Saruta T: Anti-asialo GM1 antibody suppression of cyclophosphamide-induced diabetes in NOD mice. *Diabetes Res* 1991, 17:37-41. [general reference]
173. Pearco RB, Peterson CM: Studies of concanavalin A in nonobese diabetic mice. I. Prevention of insulin-dependent diabetes. *J Pharmacol Exp Ther* 1991, 258:710-715 [general reference]

S15